[1]噪音暴露前后大鼠尿液蛋白质组的变化

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摘要

本研究建立了急性噪声暴露模型，连续 7 天每日将 10 只大鼠暴露于 119 分贝的噪声环境中，持续 9 小时，并对其尿液样本进行蛋白质组学分析。通过对比大鼠暴露前后尿液中的蛋白质表达差异，识别出 219 个差异蛋白，其中 30 个已知与听力损伤相关。特别地，Gelsolin 蛋白在调节哺乳动物耳蜗外毛细胞的机械感受毛束生长和稳定性中发挥重要作用；Lysosome-associated membrane glycoprotein 1 的缺陷在小鼠中会导致耳蜗边缘细胞的溶酶体膜蛋白空泡化和结构改变，引起听力丧失；SLIT and NTRK-like family member 6 的缺失与小鼠突触发生的延迟和听力障碍相关。这些结果表明，暴露高分贝噪音下，尿蛋白会发生一些变化，很多的变化被报道和听觉损失相关。

关键词：尿液，蛋白质组学，听力破坏，噪音暴露，尿液灵敏度

Abstract：

This study established an acute noise exposure model in which 10 rats were exposed to a 119 dB noise environment for 9 hours daily over a period of 7 consecutive days. Proteomic analysis was performed on urine samples collected from the rats. A comparison of protein expression profiles before and after noise exposure identified 219 differentially expressed proteins, 30 of which are known to be associated with hearing loss. Notably, Gelsolin plays a critical role in regulating the growth and stability of the mechanosensory hair bundles in mammalian cochlear outer hair cells. Deficiency of Lysosome-associated membrane glycoprotein 1 has been reported to cause vacuolization and structural alterations in lysosomal membrane proteins in cochlear marginal cells, leading to hearing loss in mice. Similarly, SLIT and NTRK-like family member 6 deficiency is linked to delayed synaptogenesis and auditory dysfunction in mice. These findings indicate that high-decibel noise exposure induces significant changes in urinary protein expression, many of which are associated with auditory damage as previously reported.

Keywords：urine; proteomics; Damage to hearing ; Noise exposure ; Sensitivity of urine

前言

世界卫生组织（WHO）近期公布的一项研究显示，全球范围内有高达 10 亿的 12 至 35 岁年轻人正面临听力受损的严峻风险，这一危机主要源自他们长时间且过度地暴露于高音量的音乐和其他娱乐声音之中。随着我国城市化与工业化步伐的加速，噪声污染问题愈发凸显，特别是在军工厂、机场、娱乐场所等噪音密集的区域，工作人员和访客均承受着巨大的听力受损风险，噪声性耳聋已成为不容忽视的听力致残主因。

在听力损失的诊断领域，暂时性阈移（TTS）与永久性阈移（PTS）是两种常见的状况。

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尽管组织学方法通过对突触标记的定量计数被视为诊断的金标准，但其复杂性和侵入性使得这一方法在临床实践中难以广泛应用。

传统上，听力损失的研究多依赖于组织切片、从耳部组织或细胞中提取总 RNA、总蛋白等手段。然而，一个前所未有的研究领域——尿液蛋白质组学，正逐渐崭露头角。该领域在生物标志物的发现与分析上展现出了非凡的潜力，尤其是在听力损失这一领域，此前几乎无人涉足。尿液作为生物标志物来源的独特优势在于，它不受生理稳态机制的严格束缚，因此能够更敏锐地捕捉到体内细微的生物化学波动。更重要的是，尿液的采集过程既无创又便捷，极大地提高了其在生物标志物研究中的实用性和可接受度。

目前，已有众多科学研究证实了尿液中的蛋白质能够作为多种脑部神经疾病的生物标志物。本研究正是基于这一背景，旨在通过尿液蛋白质组学的视角，深入探究噪音对听力的破坏机制。这一创新性的研究路径不仅有望为我们揭示噪音损害听力的新机制，更为开发针对此类听力障碍的新型疗法提供了前所未有的契机。

2. 材料与方法

2.1 实验动物及模型建立

SPF 级的雄性 Wistar 大鼠 (170−190 g) 20 只 ，购于北京维通利华实验动物技术有限公司，所有动物在标准环境（室温 22±1 ℃，湿度 65%–70%）下饲养，动物实验通过北京师范大学生命科学学院伦理委员会的审查和批准，编号：CLS-EAW-2020-034。模型建立的方法：将 20 只鼠随机分为两组，将其中 1 组 10 只大鼠进行噪音刺激，暴露于 119 分贝的噪音中 9 小时，持续 7 天。另外 10 只则在正常条件下饲养。

2.2 尿液样品的处理

在实验开始前，将实验组放入大鼠代谢笼中过夜收尿，对实验组进行噪音刺激后将实验组和对照组均放入大鼠代谢笼中过夜收尿。

对尿液样本进行处理时，首先将其在 4℃条件下以 12,000g 的离心力离心 30 分钟，随后使用三到五倍体积的乙醇在-20℃下沉淀每份 15 毫升尿液样本中的成分，此过程需过夜完成。次日，再次以 12,000g 离心，所得蛋白质沉淀物被溶解于裂解缓冲液中，该缓冲液包含 8 mol/L 尿素、2 mol/L 硫脲、50 mmol/L Tris 以及 25 mmol/L 二硫苏糖醇。最后，利用 Bradford 分析法对上清液中的蛋白质含量进行定量分析。

接下来，使用胰蛋白酶对总计 100 微克的蛋白质进行消化。将各样本中的蛋白质装入 10-kDa 的过滤装置内，先用尿素缓冲液和 25 mmol/L 的 NH4HCO3 溶液各洗涤两次，然后在 37℃下以 20 mmol/L 的二硫苏糖醇还原 1 小时。随后，在避光条件下，用 50 mmol/L 的碘乙酰胺（IAA）进行 45 分钟的烷基化处理。处理完毕后，再次用 UA 和 NH4HCO3 洗涤样本，并以胰蛋白酶（酶与蛋白质的比例为 1:50）在 37℃下消化过夜，持续时间约为 14 至 16 小时。消化后的肽段通过 Oasis HLB 试剂盒进行脱盐处理，并最终通过冻干机进行干燥处理。

为了进行后续分析，将干燥的肽段重新溶解于 0.1%的甲酸溶液中，并稀释至每毫升含 0.5 微克的浓度。为了构建用于数据独立采集（DIA）分析的谱图库，将混合样本（每份样本 1~2 微克）加载到平衡后的高 pH 反相分馏离心柱上。随后，向柱中加入含有 8 个不同乙腈浓度（5%、7.5%、10%、12.5%、15%、17.5%、20%和 50%乙腈）的挥发性高 pH 洗脱溶液，形成阶梯梯度，以洗脱出 8 种不同梯度组分的肽段。洗脱后的样本通过真空蒸发法进行干燥处理，并重新悬浮于 20 微升的 0.1%甲酸溶液中。最终，从每种组分中各取 2 微升用于液相色谱-数据依赖采集-串联质谱（LC-DDA-MS/MS）分析。

2.3 数据分析

通过反相色谱柱分离出的 10 个组分，采用 DDA（数据依赖性采集）模式采集质谱数据，随后把 DDA 采集所得结果导入 Proteome Discoverer 软件（版本 2.1）进行搜库操作。以 PD 搜库结果为依据建立 DIA（数据非依赖性采集）采集方法，依据 m/z 分布密度计算窗口宽度与数量。针对单个多肽样品，运用 DIA 模式采集质谱数据。采用 Spectronaut X 软件处理和分析质谱数据，导入每个样本 DIA 采集的 raw 文件进行搜库。高度可信蛋白的判定标准为肽段 q value 小于 0.01，并采用二级肽段所有碎片离子峰面积来进行蛋白定量。

2.4 统计学分析

每个样品均进行 3 次技术重复，将得到的数据用于统计学分析。针对听噪音前后的尿液蛋白进行差异蛋白筛选，筛选差异蛋白的条件为：Fold change 大于等于 1.5 或者小于等于 0.67，双尾配对 t 检验的 P 值小于 0.05。在随机分组分析中，将 20 个样本随机分成两组，在所有随机组合里，按照相同的筛选条件计算所有随机次数的平均差异蛋白数目。

3.结果与讨论

3.1 尿蛋白组变化

在噪音处理前后，对所收集到的 20 个尿液蛋白样品进行 LC—MS/MS 串联质谱分析。总共鉴定到 1552 个蛋白（特异性多肽≥2 个，蛋白水平 FDR<1%），将其进行非监督聚类分析，从这些数据中，可以区分噪音处理前后的尿液样本。图 1 展示了具体的样本非监督聚类结果。

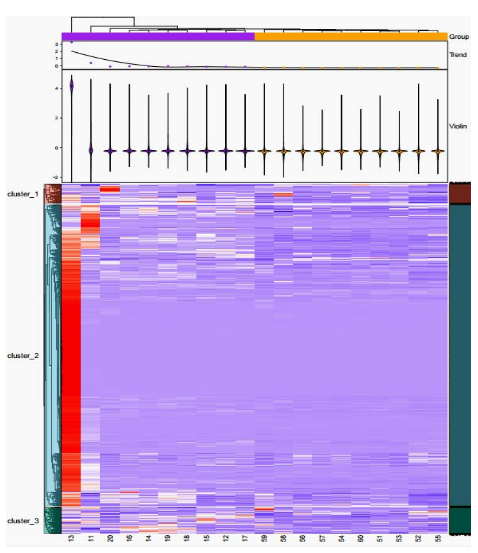


Figure 1: Unsupervised clustering

3.1 差异蛋白筛选

将噪音前后的蛋白比较，共鉴定到 219 个差异蛋白（见附录），随机分组产生的差异蛋白为 15 个，如表一所示。差异蛋白筛选条件为 FC≥1.5 或≤0.67，P < 0.05，差异蛋白的具体信息列在表 2 中。通过将分组打乱进行随机匹配得到平均差异蛋白为 15 个，证明差异蛋白结果可信，非随机产生，如表 2。

Table 1 Random grouping

Fitter Criteria	Number of differential proteins	Average numbers of proteins withfalse random combinations	Reliability
FC>1.5 或<0.67- P<0.05	219	15	93.2%

3.2 差异蛋白检索

自身前后对照通过开放数据库(https://pubmed.ncbi.nlm.nih.gov/)检索蛋白，发现 30 个与听力相关的蛋白如表 3

Table 2 Differential Proteins

Protein ID	Protein name	FC	P	
F1LQQ8	Beta-glucuronidase	3.220	0.002	[1]
P14562	Lysosome-associated membrane glycoprotein 1	1.646	0.019	[2]
A0A0G2JY48	Receptor protein-tyrosine kinase	0.619	0.010	[3]
A0A140TAI2	Biotinidase	0.607	0.020	[4]
D3ZPC4	L1 cell adhesion molecule	0.593	0.015	[5]
P20961	Plasminogen activator inhibitor 1	0.586	0.019	[6]
Q6P762	Alpha-mannosidase	0.580	0.002	[7]
P15473	Insulin-like growth factor-binding protein 3	0.579	0.019	[8]
Q08406	Ciliary neurotrophic factor receptor subunit alpha	0.556	0.002	[9]
A0A0G2JY48	receptor protein-tyrosine kinase	0.548	0.001	[10]
P98158	Low-density lipoprotein receptor-related protein 2	0.527	0.001	[11]
G3V7K2	LIF receptor subunit alpha	0.505	0.009	[9]
A0A0H2UHL7	Interleukin 1 receptor type 2	0.496	0.011	[12]
Q75NI5	Cadherin 15	0.490	0.017	[13]
B2B9A9	Ephrin B2	0.481	0.040	[14, 15]
P50430	Arylsulfatase B	0.481	0.0001	[16]
Q68FP1	Gelsolin	0.472	0.011	[17]
A0A0G2JXZ9	protein-tyrosine-phosphatase	0.458	0.007	[18]
E9PSU8	Ig-like domain-containing protein	0.438	0.013	[19]
P07483	Fatty acid-binding protein	0.393	0.022	[20]
M0RDA4	receptor protein-tyrosine kinase	0.361	0.011	[10]
D3ZGF1	CD44 antigen	0.361	0.006	[21]
A0A0G2K7Q2	Aquaporin-2	0.339	0.019	[22]
P02767	Transthyretin	0.295	0.036	[23]
M0R936	Ig-like domain-containing protein	0.294	0.018	[19]
P01836	Ig kappa chain C region, A allele	0.262	0.035	[11]
B4F795	Choline transporter-like protein 2	0.245	0.004	[24]
F1LM54	Fibroblast growth factor receptor	0.238	0.005	[25]
D3ZHG3	Protein tyrosine kinase 7	0.230	0.002	[26]

D3ZJF9	Alpha-galactosidase	0.218	0.035	[27]
D3ZP44	SLIT and NTRK-like family, member 6	0.151	0.002	[28]

Biotinidase 生物素酶缺乏症导致听力缺失[4]，Lysosome-associated membrane glycoprotein 1 L1 细胞粘附分子发布在小鼠耳蜗 Corti 器官中[5]，Alpha-mannosidase α-甘露糖苷酶活性缺陷引起听力缺陷[7]，Insulin-like growth factor-binding protein 3，缺乏 IGF-结合蛋白-3 导致听力和学习缺陷[8]，Low-density lipoprotein receptor-related protein 2 低密度脂蛋白受体相关蛋白 2 是前庭神经鞘瘤相关听力损失中的生物标志物，Interleukin 1 receptor type 2 在内耳中表达，过量表达 IL1R2 减少了噪声诱导的带状突触损伤和听力损失[12]。protein-tyrosine-phosphatase 蛋白酪氨酸磷酸酶 1B 功能丧丧导致耳聋[18]，receptor protein-tyrosine kinase 缺乏胶原蛋白受体 DDR1 的小鼠的内耳缺陷和听力损失[10]，Ig-like domain-containing protein 样结构域中的新型 ILDR1 突变导致内耳三细胞紧密连接破坏而导致的高频听力损失[19]，Protein tyrosine kinase 7 声音暴露和衰老对声音暴露后 80 天酪氨酸激酶 B 受体水平变化[26]。

本研究采用了液相色谱串联质谱（LC-MS/MS）技术，首次从尿液中寻找听力损失的线索。对暴露于噪音环境前后的大鼠尿液蛋白质组进行了全面而深入的对比分析。实验结果显示，在噪音暴露前后大鼠的尿液蛋白质组中，存在多个显著差异的蛋白质，其中多种被鉴定为与听力功能直接相关的蛋白质。这些蛋白质包括但不限于：Beta-glucuronidase、Lysosome-associated membrane glycoprotein 1、Receptor protein-tyrosine kinase、Biotinidase、L1 cell adhesion molecule、Plasminogen activator inhibitor 1、Alpha-mannosidase、Insulin-like growth factor-binding protein 3、Ciliary neurotrophic factor receptor subunit alpha、receptor protein-tyrosine kinase、Low-density lipoprotein receptor-related protein 2、LIF receptor subunit alpha、Interleukin 1 receptor type 2、Cadherin 15、Ephrin B2、Arylsulfatase B、Gelsolin、protein-tyrosine-phosphatase、Ig-like domain-containing protein、Fatty acid-binding protein、receptor protein-tyrosine kinase、CD44 antigen、Aquaporin-2、Transthyretin、Ig-like domain-containing protein、Ig kappa chain C region, A allele、Choline transporter-like protein 2、Fibroblast growth factor receptor、Protein tyrosine kinase 7、Alpha-galactosidase、SLIT and NTRK-like family, member 6。实验数据听力受损确实显示能够在尿液蛋白质组中反映出相应的变化，这些与听力功能密切相关的差异蛋白质不仅为我们提供了听力障碍发生发展的分子机制线索，同时也为未来开发针对听力障碍的有效治疗方法提供了潜在的靶点。这一发现不仅加深了我们对噪音性听力损伤分子机制的理解，也为听力障碍的早期诊断与干预开辟了新的研究方向。 Arylsulfatase B 变异引起的听力损失[16]。 gelsolin 在调节哺乳动物耳蜗外毛细胞机械感觉毛束的生长和稳定性方面具有互补作用[17]。FABP7 缺乏可减轻噪声暴露后的耳蜗损伤[20]。小鼠 DDR1 基因的缺失与听觉功能的严重下降和内耳的重大结构改变有关[10]。而 CD44 在内耳上皮中表达，与听力直接相关[21]。水通道蛋白 2 在发育中的小鼠内耳中显示出早期和特异性的表达模式，表明这种水通道蛋白在听力的发育中起重要作用[22]。转甲状腺素蛋白淀粉样变性伴头痛、听力损失和周围神经病变[23]。LC44A2（溶质载体 44a2），也称为 CTL2（胆碱转运蛋白样蛋白 2），在耳蜗的许多支持细胞类型中表达，与毛细胞存活和抗体诱导的听力损失有关[24]。Slitrk6 缺陷动物的突触发生延迟，突变小鼠表现出反映人类表型的听觉功能缺陷[28]。

而在与未听噪音的 10 只大鼠对比，共鉴定到 46 个差异蛋白，但随机分组产生的差异蛋白为 49 个，在数目上无明显差别。可能原因是个体差异的影响或生活环境对大鼠本身的影响。但在 46 个差异蛋白中仍然发现与听力损失直接相关的蛋白质。其原因可能是个体差异的影响或生活环境对大鼠的影响。

Table 2 Differential Proteins

Protein name	FC	P	
Frizzled-6	2.537	0.0011	[29]
Proteoglycan 4	2.441	0.0195	[30]
Apolipoprotein N	2.251	0.0028	[30]
Calbindin	2.0387	0.0169	[31]
Macrophage stimulating 1	1.7432	0.015	[32]
Serine protease 30	1.7390	0.040	[33]
Nectin cell adhesion molecule 3	1.5691	0.025	[34]
Fractalkine	1.5086	0.047	[35]
Annexin A8	0.5809	0.049	[36]
Ephrin-A1	0.5228	0.047	[37]

Frizzled 6 调节小鼠 Wnt 信号通路，而 Wnt 信号通路调节耳蜗毛细胞再生[29]。Proteoglycan 4 的缺失突导致触前和突触后元件的空间耦合受损，导致听力损失[30]。载脂蛋白和感音神经性听力损失存在直接关联[38]。 钙结合蛋白在听觉传导过程中调节神经元的钙离子浓度。α-1 型 XI 型胶原体的基因突变引起 2 型斯蒂克勒综合征，该基因已在小鼠的玻璃体、软骨和耳蜗中发现。该病的特征是典型的眼部异常、听觉功能障碍[39]。耳蜗巨噬细胞调节耳蜗炎症，可能具有保护听力功能免受损伤的潜力，包括声学过度刺激。耳蜗巨噬细胞数量在声学刺激后 3-7 天增加[32]。在耳蜗听觉上皮中，感觉毛发和支持细胞以棋盘状马赛克图案排列，这在广泛的物种中是保守的。细胞粘附分子 nectin-1 和 nectin-3 是这种模式形成所必需的。棋盘状模式被认为是听觉功能所必需的，但从未被检查过。在这里，我们展示了棋盘状细胞模式在 nectin-3 敲除（KO）小鼠耳蜗听觉上皮中感觉毛细胞的存活和功能中的重要性。Nectin-3 KO 小鼠表现出与通过凋亡异常附着的毛细胞退化相关的进行性听力损失。凋亡性毛细胞死亡是由于毛细胞之间紧密连接的混乱所致。我们的研究表明，听觉上皮中棋盘状的细胞模式为确保耳蜗毛细胞的存活和听力功能提供了结构基础[34]。Fractalkine 在神经系统中具有调节作用，可能影响听觉神经元的功能或保护[35]。使用膜联蛋白 A1 衍生肽进行药物治疗可预防顺铂诱导的听力损失[36] EphA2 通过与 pendrin 相互作用，调控其在内耳中的定位，影响听力功能。其突变或功能障碍可能导致 Pendred 综合征和前庭导水管扩大（EVA）患者的听力损失[37]。

4.结论

本研究首次采用了蛋白质组技术，尿液蛋白质组中探究噪音暴露对大鼠的影响。实验结果显示，在噪音暴露前后大鼠的尿液蛋白质组中，存在多个显著差异的蛋白质，其中多种曾被报道是与听力功能直接相关的蛋白质。尿蛋白质组是研究听力的新窗口。

附录 1

Protein ID	Protein name	FC	P
A0A0G2K5E1	Proline rich 4	4.09	0.001
F1LQQ8	Beta-glucuronidase (EC 3.2.1.31)	3.22	0.002
P01039	Cystatin-A	3.11	0.019
F7EZQ4	Fc fragment of IgG binding protein-like 1	2.74	0.011

D4A5U3	Protein-glutamine gamma-glutamyltransferase	2.55	0.018
Q99041	Protein-glutamine gamma-glutamyltransferase 4	2.33	0.039
Q9QYU9	Odorant binding protein I f	1.83	0.018
P14562	Lysosome-associated membrane glycoprotein 1	1.65	0.019
P97603	Neogenin	0.63	0.013
P08592	Amyloid-beta precursor protein	0.63	0.020
P45479	Palmitoyl-protein thioesterase 1	0.62	0.009
P10252	CD48 antigen	0.62	0.049
G3V6K6	Receptor protein-tyrosine kinase	0.62	0.010
A0A140TAI2	Biotinidase	0.61	0.020
Q63768	Adapter molecule crk	0.61	0.010
A0A0G2JY31	Serpin family A member 1	0.60	0.009
Q64724	C-CAM4	0.60	0.022
Q5BJU0	RAS related 2	0.59	0.043
D3ZPC4	L1 cell adhesion molecule	0.59	0.015
Q91XT9	Neutral ceramidase	0.59	0.017
P20961	Plasminogen activator inhibitor 1	0.59	0.019
Q6P762	Alpha-mannosidase	0.58	0.002
P15473	Insulin-like growth factor-binding protein 3	0.58	0.019
P54759	Ephrin type-A receptor 7	0.56	0.001
P28826	Meprin A subunit beta	0.56	0.022
B5DEZ8	Plexin domain containing 2	0.56	0.000
Q08406	Ciliary neurotrophic factor receptor subunit alpha	0.56	0.002
A0A0G2K2R5	EGF containing fibulin extracellular matrix protein 2	0.56	0.002
A0A0G2JY48	receptor protein-tyrosine kinase	0.55	0.001
A0A0G2K6T9	Protocadherin 1	0.54	0.042
F1MA46	Protocadherin 12	0.54	0.018
A0A0G2JTC1	Leukocyte immunoglobulin like receptor A5	0.53	0.003
Q99J86	Attractin (Protein zitter)	0.53	0.019
P98158	Low-density lipoprotein receptor-related protein 2 (LRP-2) (Glycoprotein 330) (gp330) (Megalin)	0.53	0.001
Q99N82	Prolactin-inducible protein homolog	0.52	0.007
Q4QQW8	Putative phospholipase B-like 2	0.52	0.000
O70244	Cubilin	0.52	0.002
Q9ESS6	Basal cell adhesion molecule	0.51	0.042
G3V7K2	LIF receptor subunit alpha	0.50	0.009
P13265	Glypican-3	0.50	0.004
P05545	Serine protease inhibitor A3K	0.50	0.003
A0A0H2UHL7	Interleukin 1 receptor type 2	0.50	0.011
Q6DGG1	Putative protein-lysine deacylase ABHD14B	0.49	0.024
Q568Z6	IST1 homolog	0.49	0.032
Q75NI5	Cadherin 15	0.49	0.017
A0A0H2UHF	Orosomucoid 1	0.48	0.042

8			
B2B9A9	Ephrin B2	0.48	0.040
Q64573	Liver carboxylesterase 1F	0.48	0.004
P50430	Arylsulfatase B	0.48	0.000
Q4KLZ0	Pantetheinase	0.48	0.002
Q32KK2	Arylsulfatase A	0.47	0.000
Q68FP1	Gelsolin	0.47	0.011
F1M7U7	Tyrosine-protein kinase receptor TYRO3	0.47	0.013
P57097	Tyrosine-protein kinase Mer	0.47	0.018
F7F5P4	Interleukin 18 binding protein	0.46	0.023
P31211	Corticosteroid-binding globulin	0.46	0.022
A0A0G2JXZ9	protein-tyrosine-phosphatase (EC 3.1.3.48)	0.46	0.007
D3ZI66	Ring finger protein 149	0.46	0.036
B0BNG3	Lman2 protein	0.46	0.025
F1LML2	Ubiquitin C	0.46	0.017
Q9QZQ5	CCN family member 3	0.46	0.043
P05544	Serine protease inhibitor A3L	0.45	0.005
D3ZF96	Lymphocyte antigen 6 family member D	0.45	0.049
D3ZDH4	receptor protein-tyrosine kinase (EC 2.7.10.1)	0.45	0.001
P15978	Class I histocompatibility antigen, Non-RT1.A alpha-1 chain	0.45	0.013
A0A0G2JZL9	Carboxylesterase 1C	0.44	0.037
P10959	chitinase (EC 3.2.1.14)	0.44	0.002
A0A0G2K676	receptor protein-tyrosine kinase	0.44	0.007
B5DFD6	Vasorin	0.44	0.002
D3ZAE6	Ig-like domain-containing protein	0.44	0.013
E9PSU8	deleted	0.44	0.021
M0RC23	Meprin A subunit alpha	0.44	0.001
Q64230	Nectin cell adhesion molecule 2	0.44	0.023
A0A0G2KA71	Ig-like domain-containing protein	0.44	0.017
G3V8Z5	Stefin-2-like	0.43	0.033
F1M861	Allograft inflammatory factor 1-like	0.43	0.005
D3ZRD9	C-reactive protein	0.43	0.003
P48199	Galactocerebrosidase	0.43	0.017
G3V6H1	Acireductone dioxygenase	0.43	0.002
Q562C9	Tyrosine-protein kinase Yes	0.43	0.022
F1LM93	Di-N-acetylchitobiase	0.43	0.003
Q01460	Hemopexin	0.43	0.009
P20059	Prostaglandin F2 receptor inhibitor	0.43	0.000
F1M790	Lysosomal alpha-glucosidase	0.43	0.001
Q6P7A9	Glucosaminyl	0.42	0.022
Q6T5E1	Serine protease 8	0.42	0.003
F1MA37	SH3 domain-binding glutamic acid-rich-like protein 3	0.42	0.028

B2RZ27	Prostaglandin-H2 D-isomerase	0.42	0.004
P22057	CD84 molecule	0.42	0.033
A0A0G2K3N2	Resistin-like gamma	0.42	0.032
G3V686	Ig gamma-2A chain C region	0.42	0.024
P20760	Alpha-1-inhibitor 3	0.41	0.048
P14046	protein-lysine 6-oxidase	0.41	0.034
D4A9V5	Membrane-bound carbonic anhydrase 14	0.41	0.000
A2IBE0	EGF containing fibulin extracellular matrix protein 1	0.41	0.001
Q6AXN2	Protein disulfide-isomerase A4	0.40	0.012
G3V6T7	tissue kallikrein	0.40	0.043
G3V8H1	Dipeptidyl peptidase 2	0.40	0.000
Q9EPB1	deleted	0.40	0.001
A0A0G2JVA4	Filamin-C	0.40	0.022
A0A0H2UHR7	Thrombomodulin	0.40	0.000
O35370	Fatty acid-binding protein	0.39	0.022
P07483	Tetraspanin	0.39	0.017
Q6P9V1	Aggrecan core protein	0.39	0.000
P07897	Charged multivesicular body protein 1B2	0.39	0.007
G3V6A6	Cartilage intermediate layer protein	0.38	0.001
D3ZGB8	Heparan sulfate proteoglycan 2	0.38	0.000
F1LTJ5	Laminin subunit gamma 2	0.38	0.032
F1LRH4	Crumbs cell polarity complex component 2	0.38	0.036
D4A3W2	deleted	0.38	0.000
E9PSQ1	Cartilage oligomeric matrix protein	0.37	0.030
M0RBU0	Alpha-mannosidase (EC 3.2.1.-)	0.37	0.039
A0A0G2K8F6	Lipoprotein lipase	0.37	0.035
Q06000	Urokinase-type plasminogen activator	0.37	0.047
P29598	Apolipoprotein E	0.37	0.000
A0A0G2K151	Semaphorin 5A	0.37	0.013
A0A0G2K7J5	Fibulin-1	0.36	0.043
D3ZQ25	Matrix remodeling-associated protein 8	0.36	0.009
A0A0G2KB26	receptor protein-tyrosine kinase	0.36	0.011
M0RDA4	CD44 antigen	0.36	0.006
D3ZGF1	Fibulin 7	0.36	0.000
D3ZSY7	deleted	0.36	0.000
A0A0G2JVT5	Matrilin 3	0.35	0.046
A0A0G2JYF9	deleted	0.35	0.000
M0R7I5	Deoxyribonuclease-1	0.35	0.004
P21704	deleted	0.34	0.048
M0R4C5	Aquaporin-2	0.34	0.019
A0A0G2K7Q2	Chymotrypsin like elastase 3B	0.34	0.013

D3ZFG3	Procathepsin L	0.33	0.006
P07154	Renin receptor	0.33	0.010
Q6AXS4	Cadherin, EGF LAG seven-pass G-type receptor 2	0.33	0.013
G3V8P3	Fibronectin	0.33	0.047
F1LST1	NHL repeat containing 3	0.33	0.035
D4A2F6	Complement factor properdin	0.33	0.008
B0BNN4	Protein FAM151A	0.33	0.013
Q642A7	Ephrin type-B receptor 3	0.33	0.038
D3ZH39	Inter-alpha-trypsin inhibitor heavy chain H3	0.33	0.001
D3ZBS2	Reticulon-4 receptor-like 2	0.32	0.000
Q80WD1	Multifunctional procollagen lysine hydroxylase and glycosyltransferase LH3	0.32	0.001
Q5U367	Sialic acid acetylesterase	0.32	0.000
A0A0A0MY22	Integrin alpha 3 variant A	0.32	0.046
D3ZCG9	deleted	0.31	0.013
M0RAZ1	Mucosal addressin cell adhesion molecule 1	0.31	0.001
O70540	Receptor-type tyrosine-protein phosphatase S	0.31	0.003
Q64605	deleted	0.31	0.000
M0R816	Extracellular leucine-rich repeat and fibronectin type III domain containing 1	0.30	0.018
D3ZZ44	Oxidized purine nucleoside triphosphate hydrolase	0.30	0.000
D3ZBB2	CD276 antigen	0.30	0.032
P53369	Leucine-rich alpha-2-glycoprotein 1	0.30	0.013
Q7TPB4	Protocadherin 19	0.30	0.040
Q5I0E1	G-protein coupled receptor family C group 5 member C	0.30	0.025
A0A0G2K8I5	Transthyretin	0.29	0.036
Q3KRC4	RT1 class I histocompatibility antigen, AA alpha chain	0.29	0.002
P02767	Ig-like domain-containing protein	0.29	0.018
P16391	Acid ceramidase	0.29	0.028
M0R936	D-dopachrome decarboxylase	0.29	0.016
A0A0G2K8T0	Neurotrimin	0.29	0.009
P80254	Protocadherin 18	0.29	0.004
Q62718	Charged multivesicular body protein 2B	0.28	0.037
A0A0G2K1P2	Ig-like domain-containing protein	0.28	0.006
F7EKI515	Amyloid P component, serum	0.28	0.044
F1M8B7	Receptor tyrosine kinase-like orphan receptor 1	0.27	0.034
F1LTN6	Adhesion G protein-coupled receptor E5	0.27	0.038
A0A0H2UHH2	Insulin-like growth factor binding protein, acid labile subunit	0.27	0.002
D3ZZ97	GTP-binding nuclear protein Ran	0.26	0.033
A0A0G2JSI4	Hyaluronidase	0.26	0.003
A0A0G2JTG4	Ig kappa chain C region, A allele	0.26	0.035

F1LRE2	N(4)-(Beta-N-acetylglucosaminyl)-L-asparaginase	0.26	0.005
P62828	Growth differentiation factor 15	0.26	0.044
F1M963	Procollagen C-endopeptidase enhancer 1	0.26	0.020
P01836	Retinol-binding protein 4	0.25	0.007
P30919	Nidogen-2 (NID-2)	0.25	0.036
G3V8K5	Beta-1,4-galactosyltransferase	0.25	0.003
O08628	receptor protein-tyrosine kinase	0.25	0.027
P04916	Choline transporter-like protein 2	0.25	0.004
B5DFC9	Beta-defensin 1	0.24	0.013
G3V722	Kazal-type serine peptidase inhibitor domain 1	0.24	0.000
A0A0G2K2H0	Polypeptide N-acetylgalactosaminyltransferase	0.24	0.017
B4F795	Hemojuvelin BMP co-receptor	0.24	0.002
O89117	Fibroblast growth factor receptor	0.24	0.005
Q3ZAU4	Cellular communication network factor 4 (Wisp1 protein)	0.24	0.011
A0A0G2JU25	6-phosphogluconolactonase (6PGL)	0.24	0.048
Q5FWU4	Protein tyrosine kinase 7	0.23	0.002
F1LM54	C-C motif chemokine 6	0.23	0.041
G3V6X0	Fibrillin 1	0.23	0.010
G3V8D5	Angiopoietin-like 2	0.23	0.013
D3ZHG3	Layilin	0.22	0.022
Q68FP3	Complement C8 gamma chain	0.22	0.030
G3V9M6	Alpha-galactosidase	0.22	0.035
G3V862	Chondroitin sulfate proteoglycan 4	0.22	0.001
A0A0G2K085	Protease, serine, 3B	0.21	0.025
D3ZPI8	Cathepsin Z	0.21	0.006
D3ZJF9	Adiponectin, C1Q and collagen domain containing	0.21	0.009
F1LS79	Serine (or cysteine) proteinase inhibitor, clade A, member 3C	0.21	0.000
G3V7Q8	Collagen type IV alpha 5 chain	0.21	0.037
Q9R1T3	deleted	0.21	0.010
A0A0G2K845	Stanniocalcin-1 (STC-1)	0.20	0.002
A0A0G2JSK1	Ephrin-A1	0.20	0.001
F1LUN5	Regenerating islet-derived protein 3-gamma (REG-3-gamma)	0.20	0.021
A0A0G2JV42	EPH-related receptor tyrosine kinase ligand 7	0.19	0.017
P97574	Protein-glucosylgalactosylhydroxylysine glucosidase	0.19	0.001
P97553	CEA cell adhesion molecule 19	0.18	0.036
P42854	Secreted Ly6/Plaur domain containing 2	0.17	0.033
A0A0G2K5X1	Endosialin	0.16	0.031
G3V989	Complement component factor h-like 1	0.16	0.049
D3ZY02	Chromosome X open reading frame, human CXorf65	0.16	0.006

	(Interleukin 2 receptor, gamma)		
D3ZE93	RCG64257-like	0.15	0.001
D3ZUR5	Ig-like domain-containing protein	0.15	0.004
D3ZN06	SLIT and NTRK-like family, member 6	0.15	0.002
Q5I0M3	Leucine-rich repeat-containing protein 15	0.14	0.028
Q68FU6	L-lactate dehydrogenase A chain	0.14	0.049
F1M3Y4	Interleukin-11 receptor subunit alpha	0.13	0.012
A0A0G2K828	Eosinophil-associated, ribonuclease A family, member 1	0.11	0.045
D3ZP44	Regenerating family member 3 beta	0.09	0.016
Q8R5M3	C-X-C motif chemokine ligand 12	0.06	0.033

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